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# Randomized, Double-Blind, Placebo-Controlled Study of Carvedilol on the Prevention of Nitrate Tolerance in Patients With Chronic Heart Failure

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***Objectives.*** **This study was designed to evaluate the effect of carvedilol on nitrate tolerance in patients with chronic heart failure.**

***Background.*** **The attenuation of cyclic guanosine 5′-monophosphate (cGMP) production due to inactivation of guanylate cyclase by increased superoxide has been reported as a mechanism of nitrate tolerance. Carvedilol has been known to combine alpha/beta-blockade with antioxidant properties.**

***Methods.*** **To evaluate the effect of carvedilol on nitrate tolerance, 40 patients with chronic heart failure were randomized to four groups that received either carvedilol (2.5 mg once a day [carvedilol group, n = 10]), metoprolol (30 mg once a day [metoprolol group, n = 10]), doxazosin (0.5 mg once a day [doxazosin group, n = 10]) or placebo (placebo group, n = 10). Vasodilatory response to nitroglycerin (NTG) was assessed with forearm plethysmography by measuring the change in forearm blood flow (FBF) before and 5 min after sublingual administration of 0.3 mg NTG, and at the same time blood samples were taken from veins on the opposite side to measure platelet cGMP. Plethysmography and blood sampling were obtained serially at baseline (day 0); 3 days after carvedilol, metoprolol, doxazosin or placebo administration (day 3); and 3 days after application of a 10-mg/24-h NTG tape concomitantly with carvedilol, metoprolol, doxazosin or placebo (day 6).**

***Results.*** **There was no significant difference in the response of FBF (%FBF) and cGMP (%cGMP) to sublingual NTG on day 0 and day 3 among the four groups. On day 6, %FBF and %cGMP were significantly lower in the metoprolol, doxazosin and placebo groups than on day 0 and day 3, but these parameters in the carvedilol group were maintained.**

***Conclusions.*** **These results indicated that carvedilol may prevent nitrate tolerance in patients with chronic heart failure during continuous therapy with NTG.**

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Organic nitrates are widely used in cardiovascular medicine, but their continuous administration can result in the rapid development of tolerance (1–3). The underlying mechanisms responsible for nitrate tolerance probably are multifactorial (4) and may include neurohormonal counterregulatory mechanisms (5), intravascular volume expansion (6) or intrinsic abnormalities such as desensitization of the target enzyme guanylate cyclase (7) or a decrease in nitroglycerin (NTG) biotransformation (8). Recent experimental data have demonstrated that nitrate tolerance is associated with increased vascular superoxide anion production (9). Therefore, antioxidant may be effective in the prevention of the development of nitrate tolerance. An increase in oxidative stress, due to an increase in free radicals, a relative deficiency in the endogenous antioxidant reserve or both, has been suggested to be one of the contributing factors in the pathogenesis of heart failure (10), and carvedilol has been reported to have a beneficial effect on the survival of patients with heart failure (11–13). Carvedilol has been known to combine alpha/beta-blockade with antioxidant properties (14–17). Therefore, this study was designed to investigate the effect of carvedilol on nitrate tolerance during continuous administration of NTG in patients with chronic heart failure. Moreover, to clarify the mechanism of preventive effects of carvedilol on nitrate tolerance, we compared these effects among carvedilol (alpha/beta-blocker with antioxidant), metoprolol (beta-blocker without antioxidant), doxazosin (alpha-blocker without antioxidant) and placebo.

## Methods

**Patient population.** The study population comprised 40 patients with chronic heart failure ranging in age from 45 to 69 years. Heart failure was defined as dyspnea or fatigue on exertion for ≥3 months, in association with a left ventricular ejection fraction ≤0.45 as assessed by echocardiography, and a maximum load <7 metabolic equivalents as assessed by a treadmill exercise

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**Abbreviations and Acronyms**

cGMP = cyclic guanosine 5′-monophosphate
FBF = forearm blood flow
NTG = nitroglycerin

test. The clinical status and background medications received by each patient were stable for ≥2 months before enrollment in this study. Patients were excluded from participation if they had primary valvular disease, active myocarditis or any condition other than heart failure that could limit exercise (e.g., hepatic, renal or endocrine disease). Patients receiving calcium channel blocking agents, alpha- or beta-adrenergic agonist or antagonist drugs, specific antiarrhythmic drugs or antioxidants (vitamin E, vitamin C or hydralazine) were also excluded. All medications were withdrawn >48 h before the study. Patients' characteristics are shown in Table 1.

**Study protocol.** We measured the vasodilator response to sublingual NTG by plethysmography and platelet cyclic guanosine 5′-monophosphate (cGMP) at baseline (day 0), after 3 days of administration of carvedilol (Artist; Daiichi Seiyaku, Tokyo, Japan), metoprolol SR (Lopresor; Novartis, Tokyo, Japan), doxazosin (Cardenalin; Pfizer, Tokyo, Japan) or placebo (day 3), and 3 days after the application of a 10-mg/24-h NTG tape concomitantly with carvedilol, metoprolol, doxazosin or placebo (day 6). An 18-gauge heparin lock was inserted in the contralateral forearm to allow venous blood sampling for measurements of the platelet cGMP level.

The baseline forearm blood flow (FBF) was recorded by plethysmography before and 5 min after administration of 0.3 mg of sublingual NTG on day 0. Subjects were then allocated by a double-blind parallel design to four groups that received either 2.5 mg carvedilol once a day (carvedilol group, n = 10), 30 mg metoprolol SR once a day (metoprolol group, n = 10), 0.5 mg doxazosin once a day (doxazosin group, n = 10), or placebo once a day (placebo group, n = 10). Subjects returned 3 days after treatment with carvedilol, metoprolol, doxazosin or placebo for measurements of the FBF and the platelet cGMP before and after administration of sublingual NTG. A 5-mg NTG tape (Millisrol Tape; Nippon Kayaku, Tokyo, Japan) was then applied twice a day. Final measurements were obtained 3 days after treatment with combined continuous transdermal NTG. The study protocol is shown in Figure 1.

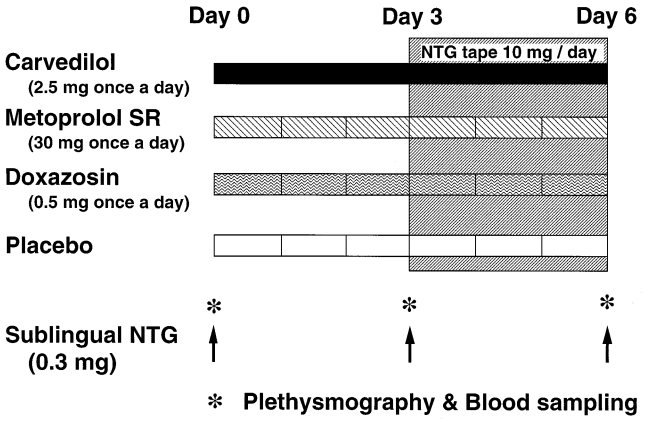


**Figure 1.** Study protocol.

The study protocol was approved by the ethics committee of KINU Medical Association Hospital, Mitsukaido, Japan, and the University of Tsukuba, Tsukuba, Japan, and written informed consent for participation in this study was obtained from all subjects.

**Assessment of the vasodilator response to NTG.** To evaluate the vasodilator response to NTG, the FBF was measured with a mercury-in-silastic strain gauge plethysmograph and the venous occlusion technique. The strain gauge was placed 5 cm below the antecubital crease and connected to a calibrated plethysmograph. The FBF is expressed as the rate of change in the forearm volume (ml/min/100 ml forearm). The pressure in

**Table 1.** Patient Characteristics

| | Carvedilol (n = 10) | Metoprolol (n = 10) | Doxazosin (n = 10) | Placebo (n = 10) |
|---|---|---|---|---|
| Age (yr) | 59 ± 8 | 57 ± 7 | 58 ± 9 | 56 ± 9 |
| Male/female | 7/3 | 6/4 | 6/4 | 7/3 |
| New York Heart Association functional class (II/III/IV) | 8/2/0 | 8/2/0 | 7/3/0 | 8/2/0 |
| Cause of heart failure | | | | |
| Coronary artery disease | 6 | 5 | 7 | 6 |
| Nonischemic dilated cardiomyopathy | 4 | 5 | 3 | 4 |
| Left ventricular ejection fraction | 0.37 ± 0.09 | 0.38 ± 0.09 | 0.35 ± 0.07 | 0.38 ± 0.08 |
| Heart rate (beats/min) | 83 ± 10 | 85 ± 10 | 84 ± 12 | 85 ± 12 |
| Systolic pressure (mm Hg) | 122 ± 15 | 125 ± 17 | 123 ± 14 | 127 ± 15 |
| Diastolic pressure (mm Hg) | 72 ± 12 | 77 ± 13 | 75 ± 11 | 76 ± 12 |
| Patients receiving digitalis | 2 | 2 | 1 | 2 |
| Patients receiving diuretics | 9 | 8 | 9 | 8 |
| Patients receiving angiotensin-converting enzyme inhibitor | 8 | 8 | 7 | 8 |
| Patients receiving vasodilator | 4 | 4 | 3 | 3 |

the venous occlusion or congesting cuff was 40 mm Hg. Circulation to the hand was arrested during determinations of FBF by inflation of a cuff around the wrist to suprasystolic pressure. We used the average of three measurements made at 15-s intervals to represent the FBF. The intraobserver and interobserver variabilities for repeated measurements are 0.12 ± 0.31 and 0.04 ± 0.10 ml/min/100 ml forearm in our laboratory.

**Preparation of platelet cGMP.** Blood samples were drawn into syringes containing 5 mM ethylenediaminetetraacetic acid and a cGMP phosphodiesterase inhibitor ($10^{-3}$ mol/L 2-*O*-propoxyphenyl-8-azapurin-6-one dissolved in 1% triethanolamine). Platelet-rich plasma and platelet-poor plasma were prepared immediately after blood sampling by centrifugation at 200 *g* for 20 min. Platelet-rich plasma was further centrifuged at 2,500 *g* for 10 min, and the supernatant was discarded. The pellet was suspended in modified Tyrode's solution (containing 0.35% bovine serum albumin and 5 mM Hepes, pH 7.35) to obtain a final platelet count of 2 to $3 \times 10^6$ platelets/$\mu$l. The samples were stored frozen at −70° C until analysis (18).

**Platelet cGMP assay.** Trichloroacetic acid (0.5 ml in a final concentration of 6%) was added to 1 ml of the platelet preparation. After centrifugation at 2,500 *g* for 20 min, trichloroacetic acid was extracted four times from the supernatant with water-saturated ether. The aqueous phase was then assayed for cGMP using a commercially available radioimmunoassay kit (Yamasa Shoyu, Choshi, Japan) (19). The results are expressed in picomoles/$10^9$ platelets. The coefficients of variation averaged 3.4% for intraassay error and 11.9% for interassay error.

**Statistical analysis.** Results are expressed as the mean ± SD for the FBF and the platelet cGMP level. Differences among the test days or differences before and after sublingual NTG were analyzed by repeated measures analysis of variance (ANOVA) with Bonferroni's test, and differences among groups were analyzed by factorial analysis of variance followed by Scheffé F test. Values of $p < 0.05$ were considered significant.

## Results

**Heart rate and blood pressure (Table 2).** Heart rate did not change during this study in the four groups. There was no difference in heart rate before and after sublingual NTG or among days 0, 3 and 6 in the four groups. Mean blood pressure was significantly decreased after sublingual NTG on days 0 and 3 in the four groups. However, in the metoprolol, doxazosin and placebo groups, mean blood pressure after sublingual NTG was not decreased on day 6. On the other hand, in the carvedilol group the change of mean blood pressure after sublingual NTG on day 6 was similar to that on day 0.

**FBF (Table 3).** The FBF increased after sublingual NTG on days 0 and 3 in the four groups. There was no significant difference in the FBF before and after NTG among the four groups. On day 6 the FBF increased in the metoprolol, doxazosin and placebo groups after sublingual NTG, but was significantly lower compared with days 0 and 3. In the carvedilol group the change in the FBF after sublingual NTG was similar to those on days 0 and 3. The NTG after sublingual NTG on day 6 was significantly greater in the carvedilol group than in the other groups.

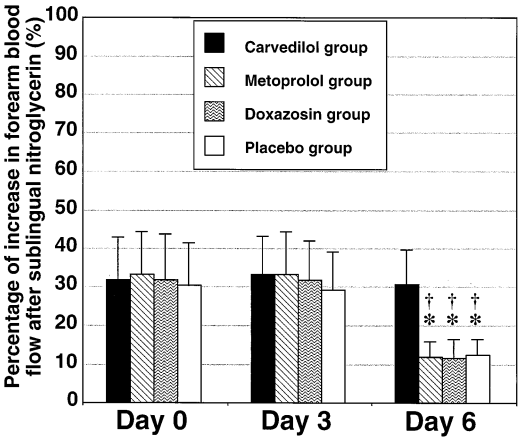


**Figure 2.** Percentage of increase in the forearm blood flow after sublingual nitroglycerin. Data are expressed as the mean value ± SD. *p < 0.05 versus days 0 and 3; †p < 0.05 versus carvedilol group.

There was no significant difference in the percent increase in the FBF on days 0 and 3 among the four groups (day 0: carvedilol group, 32 ± 11%; metoprolol group, 33 ± 11%; doxazosin group, 32 ± 12%; placebo group, 30 ± 11%; day 3: carvedilol group, 33 ± 10%; metoprolol group, 33 ± 11%; doxazosin group, 32 ± 10%; placebo group, 29 ± 10%). The percent increase in the metoprolol, doxazosin and placebo groups was significantly lower on day 6 (metoprolol group, 12 ± 4%; doxazosin group, 12 ± 5%; placebo group, 13 ± 4%) than on days 0 and 3. In the carvedilol group the percent increase in the FBF after sublingual NTG was maintained on day 6 (31 ± 9%) and was significantly greater than in the other groups (Fig. 2).

**Platelet cGMP level (Table 3).** The platelet cGMP level was significantly increased after sublingual NTG in the four groups on days 0 and 3. There was no significant difference among the four groups on days 0 and 3.

The platelet cGMP level before and after sublingual NTG on day 6 was significantly lower in the metoprolol, doxazosin and placebo groups than that on days 0 and 3, whereas the platelet cGMP level in the carvedilol group on day 6 was significantly increased after sublingual NTG, and was significantly higher than in the other groups. There was no significant difference in the platelet cGMP level in the carvedilol group among test days.

There was no difference in the percent increase in the platelet cGMP level between the carvedilol group and the placebo group on days 0 and 3 (day 0: carvedilol group, 38 ± 10%; metoprolol group, 40 ± 11%; doxazosin group, 41 ± 11%; placebo group, 39 ± 12%; day 3: carvedilol group, 39 ± 11%; metoprolol group, 40 ± 12%; doxazosin group, 40 ± 11%; placebo group, 39 ± 10%). The percent increase in cGMP level in the metoprolol,

**Table 2.** Heart Rate and Mean Blood Pressure Before and After Sublingual Administration of Nitroglycerin

| | | Day 0 | | | | Day 3 | | | | Day 6 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Carvedilol Group | Metoprolol Group | Doxazosin Group | Placebo Group | Carvedilol Group | Metoprolol Group | Doxazosin Group | Placebo Group | Carvedilol Group | Metoprolol Group | Doxazosin Group | Placebo Group |
| Heart rate (beats/min) | Before | 81 ± 11 | 81 ± 10 | 82 ± 11 | 82 ± 10 | 80 ± 10 | 78 ± 11 | 81 ± 12 | 82 ± 11 | 81 ± 11 | 79 ± 12 | 80 ± 12 | 82 ± 13 |
| | After | 85 ± 11 | 85 ± 11 | 86 ± 10 | 85 ± 12 | 81 ± 11 | 77 ± 12 | 81 ± 13 | 88 ± 13 | 82 ± 10 | 80 ± 11 | 81 ± 11 | 83 ± 11 |
| Blood pressure (mm Hg) | Before | 91 ± 12 | 93 ± 11 | 91 ± 12 | 92 ± 12 | 89 ± 10 | 88 ± 12 | 90 ± 11 | 92 ± 11 | 88 ± 12 | 89 ± 10 | 88 ± 10 | 90 ± 10 |
| | After | 86 ± 11* | 86 ± 10* | 85 ± 11* | 86 ± 11* | 84 ± 11* | 85 ± 11* | 85 ± 12* | 86 ± 12* | 84 ± 12* | 87 ± 11 | 86 ± 11 | 89 ± 12 |

Data are expressed as mean ± SD. Before = before sublingual administration of nitroglycerin; after = after sublingual administration of nitroglycerin. *$p < 0.05$ versus before.

doxazosin and placebo groups was significantly lower on day 6 (metoprolol group, 11 ± 6%; doxazosin group, 12 ± 5%; placebo group, 14 ± 5%) compared with days 0 and 3. The percent increase in the platelet cGMP level in the carvedilol group was maintained on day 6 (36 ± 9%) and was significantly greater than in the other groups (Fig. 3).

## Discussion

This placebo-controlled, double-blind study demonstrated that carvedilol maintained the response of vasodilation and the intracellular production of cGMP after sublingual NTG during transdermal application of NTG. These findings suggest that carvedilol, an alpha/beta-blocker with antioxidant properties, may prevent nitrate tolerance in patients with chronic heart failure.

**Proposed mechanisms of nitrate tolerance.** Although the phenomenon of nitrate tolerance was first described during the early part of this century (20), it was not considered clinically important (21) until later research demonstrated that nitrate tolerance limited the efficacy of these drugs in patients with ischemic heart disease and congestive heart failure (22–24). The mechanism of nitrate tolerance is multifactorial (4,23,25). Nitrate tolerance is thought to be due to the inability of the vascular tissue to respond to NTG (26). Previous studies have proposed four possible mechanisms of nitrate tolerance after chronic exposure: (1) desensitization of the target enzyme guanylate cyclase (7); (2) an increase in phosphodiesterase activity (27); (3) intracellular sulfhydryl group depletion (28); and (4) impaired NTG biotransformation (29). Moreover, Münzel et al. (9) recently demonstrated that enhanced angiotensin II activities resulted in increased production of oxygen-derived radicals, which inhibit the dilator action of NTG-derived nitric oxide.

**The effect of antioxidants on nitrate tolerance.** An increase in oxidative stress due to an increase in free radicals may be one of the contributing factors in the mechanisms of nitrate tolerance. These radicals inactivate the enzymes involved in the release of nitric oxide from NTG, leading to impaired cGMP production. Therefore, we thought that some antioxidants may prevent nitrate tolerance. However, there are some investigations that evaluated the effect of antioxidant on nitrate tolerance. In an experimental animal study, Bassenge and Fink (30) demonstrated that vitamin C prevented nitrate tolerance in the dilatation of the coronary artery and the production of platelet cGMP. Recently two studies demonstrated the preventive effect of hydralazine on nitrate tolerance (31,32). Münzel et al. (33) proposed that antioxidant properties may contribute to the preventive effect of hydralazine on nitrate tolerance. We also reported that oral supplementation of vitamins E and C could prevent nitrate tolerance in FBF and platelet cGMP in normal volunteers and patients with ischemic heart disease (34,35), and that intravenous application of vitamin C could prevent nitrate tolerance in patients with congestive heart failure (36).

**The effect of carvedilol on nitrate tolerance.** Carvedilol, in addition to being an alpha/beta-adrenergic antagonist and a

**Table 3.** Forearm Blood Flow (ml/min/100 ml arm) and Platelet cGMP Level (pmol/$10^9$ Platelets) Before and After Sublingual Administration of Nitroglycerin

| | | Day 0 | | | | Day 3 | | | | Day 6 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Carvedilol Group | Metoprolol Group | Doxazosin Group | Placebo Group | Carvedilol Group | Metoprolol Group | Doxazosin Group | Placebo Group | Carvedilol Group | Metoprolol Group | Doxazosin Group | Placebo Group |
| Forearm blood flow | Before | 2.2 ± 0.8 | 2.1 ± 0.8 | 2.2 ± 0.7 | 2.3 ± 0.7 | 2.4 ± 0.8 | 2.1 ± 0.7 | 2.5 ± 0.6 | 2.4 ± 0.7 | 2.6 ± 0.8 | 2.2 ± 0.7 | 2.3 ± 0.9 | 2.4 ± 0.6 |
| | After | 2.9 ± 0.7* | 2.8 ± 0.9* | 2.9 ± 0.7* | 3.0 ± 0.7* | 3.2 ± 0.8* | 2.8 ± 0.9* | 3.3 ± 0.7* | 3.1 ± 0.9* | 3.4 ± 0.8* | 2.5 ± 0.9*†‡ | 2.6 ± 0.7*†‡ | 2.7 ± 0.6*†‡ |
| cGMP | Before | 0.39 ± 0.16 | 0.38 ± 0.18 | 0.37 ± 0.22 | 0.41 ± 0.12 | 0.39 ± 0.25 | 0.38 ± 0.19 | 0.38 ± 0.19 | 0.39 ± 0.18 | 0.55 ± 0.19 | 0.35 ± 0.24‡ | 0.34 ± 0.27‡ | 0.36 ± 0.25‡ |
| | After | 0.54 ± 0.19* | 0.53 ± 0.23* | 0.52 ± 0.25* | 0.57 ± 0.15* | 0.54 ± 0.23* | 0.53 ± 0.24* | 0.53 ± 0.17* | 0.54 ± 0.16* | 0.75 ± 0.16* | 0.39 ± 0.17*†‡ | 0.38 ± 0.18*†‡ | 0.41 ± 0.29*†‡ |

Data are expressed as mean ± SD for forearm blood flow and cGMP. Before = before sublingual administration of nitroglycerin; after = after sublingual administration of nitroglycerin. *$p < 0.05$ versus before; †$p < 0.05$ versus day 0; ‡$p < 0.05$ versus carvedilol group.




**Figure 3.** Percentage of increase in the platelet cyclic guanosine 5′-monophosphate level after sublingual nitroglycerin. Data are expressed as the mean value ± SD. *$p < 0.05$ versus days 0 and 3; †$p < 0.05$ versus carvedilol group.

vasodilator, appears to be an antioxidant and free radical scavenger as well (14–17). In this study carvedilol prevented the attenuation in the vasodilatory response and the intracellular production of cGMP after administration of sublingual NTG during continuous transdermal application of NTG. To clarify the mechanism of the preventive effect of carvedilol on nitrate tolerance, we compared these effects with metoprolol, a beta-blocker without antioxidant, and doxazosin, an alpha-blocker without antioxidant. In our results metoprolol and doxazosin did not prevent nitrate tolerance. Therefore, we speculated that antioxidant properties of carvedilol might contribute to the preventive effect on nitrate tolerance. Carvedilol is approximately tenfold more potent as an antioxidant than vitamin E. Several metabolites of carvedilol (most notably SB-209995) are extremely potent antioxidants, being 30 to 80 times more potent than carvedilol as antioxidants and up to 1,000 times more potent than vitamin E (17,37). Moreover, the potency of carvedilol as an antioxidant was recently shown to be less than 100 pM in a rat cardiac ischemia/reperfusion model in vitro (38). To our knowledge, this study provides the first evidence in the clinical investigation that the development of nitrate tolerance may be prevented by supplementing with a small dose of carvedilol.

**Study limitations.** There are some limitations in this study. First, we measured the platelet cGMP level to evaluate the intracellular production of cGMP. The in vivo effects of NTG on the intracellular production of cGMP in the vascular smooth muscle cells can be evaluated only in biopsy specimens. Nitroglycerin activates soluble guanylate cyclase in platelets, and the increased level of platelet cGMP inhibits platelet adhesion (39,40). Platelets contain predominantly the soluble guanylate cyclase (41,42). Therefore, platelets are an appropriate material for the clinical measurement of intracellular cGMP. In a previous study we demonstrated that the platelet cGMP level can be used as an indicator of the effects of NTG and the development of nitrate tolerance (18).

Second, we evaluated the effect of a single small dosage of carvedilol (2.5 mg/day), metoprolol (30 mg/day) and doxazosin (0.5 mg/day). The doses of carvedilol and metoprolol used in this study are starting doses in the therapy of heart failure in Japan. Of course the dosage in this study was lower than that in other carvedilol studies on heart failure, and we did not observe any changes in heart rate and blood pressure before sublingual NTG among three testing days (days 0, 3 and 6) in this study. It is important to note that our results indicate that antioxidant properties of carvedilol may be effective even in small doses, which cannot provide any effects as an alpha/beta-blockade. So carvedilol may be a useful antioxidant in small doses to prevent nitrate tolerance in patients with chronic heart failure.

Third, we studied the effects of carvedilol only in patients with chronic heart failure, but did not study normal subjects. It is well known that patients with chronic heart failure are characterized by systemic vasoconstriction and a reduced peripheral perfusion due to endothelial dysfunction of peripheral resistance arteries (43). In our previous study FBF and platelet cGMP before sublingual NTG were 2.6 ± 0.7 ml/min/100 ml forearm (mean ± SD) and 0.62 ± 0.05 pmol/$10^9$ platelets (mean ± SEM) in normal volunteers (36). Our results of FBF and platelet cGMP in this study were lower than those in our previous study. These differences between the normal state and the heart failure state may be associated with baseline endothelial-dependent vasodilation (endothelial dysfunction in the heart failure state). However, the percent increase in FBF and platelet cGMP after sublingual NTG in this study were similar to those in our previous study. These results indicate that there is no difference in the effects of NTG (endothelial-independent vasodilation) in the normal state and the heart failure state.

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